American Journal of Hematology 56:93–99 (1997)

Thrombomodulin and Induced Tissue Factor Expression on Monocytes as Markers of Diabetic Microangiopathy: A Prospective Study on Hemostasis and Lipoproteins in Insulin-Dependent Diabetes Mellitus

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Vascular complications are the main cause of morbidity in diabetes mellitus. To evaluate lipoprotein and hemostatic parameters and their relationship with clinically detectable microangiopathy, we studied 58 insulin-dependent diabetes mellitus patients and 60 controls matched for age, sex, and body mass index. Thirteen patients presented clinically detectable microangiopathy (8 retinopathy and 5 both retinopathy and microalbuminuria). A cross-sectional study of lipid profile, coagulation parameters, and a flow-cytometric evaluation of tissue factor expression in normal monocytes induced by patient plasma were performed. Patients were re-evaluated for microangiopathy in a 3-year median follow-up. Patients showed triglyceride enrichment in low ($P = 0.00002$) and high density lipoproteins ($P = 0.004$) and increased levels of D-dimer ($P < 0.00001$), prothrombin fragment 1 + 2 ($P < 0.00001$), and thrombin-antithrombin III complex ($P = 0.0001$). Patients with clinically detectable microangiopathy had increased type 1 plasminogen activator inhibitor ($P = 0.00001$), thrombomodulin ($P = 0.02$), and induced monocyte tissue factor expression ($P < 0.00001$). Nine patients developed clinically detectable microangiopathy in the follow-up and the only predictive variable was increased induced tissue factor expression. In conclusion, in these patients elevated thrombin and fibrin generation reflects a hypercoagulable state but clinically detectable microangiopathy seems related to endothelial cell injury markers and to increased induced tissue factor expression on monocytes. Am. J. Hematol. 56:93–99, 1997. © 1997 Wiley-Liss, Inc.

Key words: hemostasis; lipoproteins; microangiopathy; thrombomodulin; tissue factor; monocytes

INTRODUCTION

Vascular complications are the main cause of morbidity and mortality in diabetes mellitus [1]. Although good glycemic control reduces the incidence of microvascular complications [2,3], retinopathy [4] and nephropathy [5] progress in some patients, despite optimal metabolic control, suggesting that factors other than glycemia such as abnormalities in both lipid and hemostatic parameters, may play a role in the development of diabetic microangiopathy. Thus, an altered lipoprotein profile leading to a pro-atherogenic pattern has been described in diabetes mellitus [6], and a hypercoagulable state may be an aggravating factor. However, some studies in lipid and coagulation parameters have reported controversial results, probably because of the different methodologies used and the diversity of the populations studied [7,8]. Furthermore, the relationship of these alterations with mi-

Contract grant sponsor: Fondo de Investigaciones Sanitarias de la Seguridad Social; Contract grant numbers: FIS 93/1050, FIS 94/5402, FIS 95/0250; Contract grant sponsor: Fundació Abelló i Pascual.

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Received 19 July 1996; Accepted 11 June 1997.

croangiopathy have not been broadly analyzed and studies have failed to differentiate between abnormalities due to, or predictive of, vascular lesions.

In the present study we have evaluated lipoprotein and hemostatic parameters and their relationship with clinically detectable microangiopathy in a cohort of insulin-dependent diabetes mellitus (IDDM) outpatients with stable metabolic control. Furthermore, the predictive value of these parameters in the development of clinically detectable microangiopathy was evaluated.

MATERIALS AND METHODS

Subjects

We studied 58 IDDM patients attending the outpatient clinic of the Hospital ''Germans Trias i Pujol.'' Informed consent was obtained from all the participants and the study was carried out according to the principles of the Declaration of Helsinki. There were 28 males and 30 females, aged between 15 and 55 years (mean 28.1 ± 10.9 years). Body mass index (BMI) was <30 Kg/m^2 in all cases. Patients had stable metabolic control without acute hyper- or hypoglycemic decompensations within the previous 6 months. Sixty healthy subjects matched for age, sex, and BMI were included as controls. None of the patients or controls had taken steroids, β-blockers, hormonal contraceptives, aspirin, or other drugs that may affect lipid metabolism or hemostasis. None had an alcohol intake greater than 20 g a day. None of the females was pregnant. All the patients and controls had normal liver function.

Methods

Clinical data were obtained from medical records. Data evaluated were blood pressure, glycemia, glycosilated hemoglobin (HbA_{1c}) and diabetic complications. Nephropathy was evaluated by serum creatinine and 24-hr urinary albumin excretion. Microalbuminuria was considered when urinary albumin excretion was between 30 and 300 mg/24 hr. Retinopathy was studied by direct fundoscopy and by fluorescein angiography. A patient was considered to have clinically detectable microangiopathy if retinopathy and/or microalbuminuria was present. Macroangiopathy was evaluated by electrocardiography and oscillometry and recording clinical data of stroke and coronary insufficiency. A complete neurological examination was performed to define the presence of neuropathy. Table I summarizes the patients' clinical characteristics. Thirteen patients had clinically detectable microangiopathy (8 retinopathy and 5 both retinopathy and microalbuminuria) but none had macroangiopathy.

Patients were re-evaluated every 6 months for micro- and macroangiopathy using the above-mentioned criteria during a median follow-up of 3 years from inclusion in the study (range: 2.0–3.5 years).

TABLE I. Clinical Data of IDDM Patients and Controls*

	IDDM (n = 58)	Controls (n = 60)
Age (years)	28.1 ± 10.9	28.4 ± 11.2
Sex (male/female)	28/30	29/31
Body mass index (kg/m^2)	24.2 ± 2.8	24.0 ± 2.7
Diabetes duration (months)	94.6 ± 68.8	—
Basal glycemia (mmol/L)	8.8 ± 2.7	4.6 ± 0.4
HbA_{1c} (%)	7.6 ± 1.3	—
Blood pressure (mmHg)		
Systolic	120 ± 18	118 ± 20
Diastolic	69 ± 11	70 ± 12
Sensitive neuropathy (%)	3 (5.2)	0
Retinopathy (%)	13 (22.4)	0
Microalbuminuria (%)	5 (8.6)	0
Smokers (%)	9 (15.5)	11 (18.3)

*IDDM: Insulin-dependent diabetes mellitus; HbA_{1c}: glycosilated hemoglobin.

Venous blood samples were drawn from a clean antecubital venipuncture without venocclusion, with the patient sitting and resting, in the morning after an overnight fasting. Blood samples were immediately transferred to sterile glass tubes without additives (Becton Dickinson, Rutherford, NJ) for lipoprotein studies, to EDTA added tubes (Becton Dickinson) for HbA_{1c} determination, and to polypropylene tubes containing 3.8% trisodium citrate (1:9, vol:vol) (Becton Dickinson) for hemostasis studies. Glucose and creatinine were determined by routine autoanalyzer methods. HbA_{1c} was evaluated by spectrophotometric chromatography in ionic exchange microcolumn (Biosystem, Barcelona, Spain). Urinary albumin concentration was estimated by nephelometry (interassay coefficient of variation = 2.0%).

Lipoprotein Studies

Lipoprotein isolation was carried out as follows: The blood was allowed to clot for 1 hr at room temperature, the serum was removed by centrifugation (1,200*g*, 20°C, 15 min), supplemented with a preservative solution, and stored at 4°C for no more than 4 days before ultracentrifugation. Lipoprotein isolation was carried out by a double ultracentrifugation procedure [9]. Briefly, we overlayered serum with NaCl solution (density = 1.006 kg/L) and centrifuged the samples in a fixed-angle 50.38 Ti rotor (Kontron Instruments, Milan, Italy) at 150,000*g* and 10°C for 18 hr. Very-low-density lipoprotein (VLDL) fraction was collected by aspiration from the top of the tube. The infranatant was adjusted to density = 1.25 kg/L with dried KBr and overlayered sequentially with a density = 1.21 kg/L salt solution and distilled water. The samples were then ultracentrifuged in a TST 41.14 rotor (Kontron Instruments) at 300,000*g* for 22 hr, and the other lipoproteins [intermediate-density lipoproteins (IDL) $1.006 < d < 1.019$, low-density lipoproteins (LDL) $1.019 < d < 1.063$, and high-density lipoproteins

(HDL) d > 1.063] were aspirated. Cholesterol and triglycerides were assayed by enzymatic methods. The mean intra- and inter-series coefficients of variation for the double ultracentrifugation procedure never exceeded 10%. Proteins of each isolated fraction were measured by a colorimetric method. Apolipoprotein (apo) E polymorphism was studied in patients and controls by isoelectric focusing from delipidated VLDL as described previously [10]. Lipoprotein(a) was quantified by enzyme linked immunoanalysis (ELISA) (TintElize Lp(a), Biopool, Umeä, Sweden).

Hemostasis Studies

Platelet-free plasma was obtained by centrifugation, aliquoted, snap-frozen in dry ice/ethanol mixture, and stored at −70°C. Both prothrombin and activated partial thromboplastin time (aPTT) were determined in an automated MLA Electra 1000C system (Medical Laboratory Automation Inc., Pleasantville, NY) using standard reagents (Thromboplastin IS and Actin FS; Dade, Miami, FL) and were expressed as ratios (patient time/control time). Fibrinogen was measured by Clauss's technique [11]. Factor VII was determined with one stage clotting assay using rabbit brain thromboplastin (Thromboplastin IS) and factor VII deficient human plasma (Dade). Antithrombin III activity was measured using Behrichrom Antithrombin III kits (Behringwerke, Marburg, Germany) [12]. Protein C activity was quantified by a colorimetric assay (Chromogenix, Mölndal, Sweden) [13]. Antigen related to tissue-type plasminogen activator (t-PA) was measured by means of an ELISA (Stago, Asnières, France) [14]. Type 1 plasminogen activator inhibitor (PAI-1) activity was measured by the method described by Chmielewska et al. (Chromogenix) [15]. Plasminogen was evaluated using a chromogenic assay (Chromogenix) [16]. Plasma levels of prothrombin fragment 1 + 2 (F_{1+2}) [17], thrombin-antithrombin complex (TAT) [18], and D-dimer [19] were assessed by ELISA techniques (Enzygnost-F_{1+2} and Enzygnost-TAT, Behringwerke; and ELISA D-dimer, Stago). Thrombomodulin was measured by an ELISA method using two monoclonal antibodies directed against the extracytoplasmatic NH_2-terminal domain [20] (Stago).

Induced Tissue Factor Expression on Monocytes

Induced tissue factor expression on normal monocytes was performed as previously described [21]. Briefly, blood anticoagulated with CPD (Citrate-Phosphate-Dextrose, final concentration of citrate 19 mmol/L) was obtained from blood group O normal volunteers and mononucleated blood cells isolated by sedimentation on Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden). After washing in phosphate buffered saline (PBS), pH 7.2 (Bio-Mérieux, Marcy-l'Etoile, France), mononucleated blood cells were incubated (6 hr, 37°C) in plasma samples from patients or controls (20×10^6 cells/mL). Cell viability determined by the trypan blue exclusion technique was greater than 95% in all the preparations and all the plasma samples and supernatants of incubations showed negligible amounts of endotoxin in a Limulus Amoebocyte Lysate test (Chromogenix). Positive controls were obtained incubating mononucleated blood cells with control plasma plus 10 μg/mL lipopolysaccharide (Difco, Detroit, MI).

After incubations, mononucleated blood cells were washed twice with PBS, pH 7.2, plus 1% bovine serum albumin (Sigma, St Louis, MO) and 1% heat inactivated human serum AB, and incubated (30 min, 22°C) with a phycoerythrin-tagged monoclonal antibody anti CD14 (LeuM3; Becton Dickinson) and with a fluorescein-tagged monoclonal antibody against tissue factor (American Diagnostic, Greenwich, CT). Nonspecific binding was assessed with an irrelevant isotype-matched fluorescein-labelled monoclonal antibody. Flow cytometry was performed on a FACScan (Becton Dickinson) using Lysys II software (Becton Dickinson). Monocytes were monitored using forward scatter, side scatter, and 585 nm fluorescence (phycoerythrin) gates. Tissue factor expression was read at 530 nm (fluorescein) and data of $5–10 \times 10^3$ monocytes collected in logarithmic amplification. Quadrant analysis of two-color dot-plot histograms was performed by setting the horizontal and the vertical cursor on the isotypic negative control population to ensure there was less than 0.5% positive cells. Percentage of tissue factor positive monocytes (%TF) and mean channel of fluorescence intensity (MFI) expressed as arbitrary fluorescence units (afu) was recorded.

Tissue factor expression on normal monocytes prior to incubation with plasmas was low (MFI: 8.0 ± 2.4 afu, %TF: 7.9 ± 3.1%). Incubation with control plasmas did not show any significant effect (MFI: 8.7 ± 4.0 afu, %TF: 8.3 ± 3.5%) whereas lipopolysaccharide addition caused a clear increase (MFI: 45.6 ± 16.8 afu, %TF: 56.7 ± 17.9%, $P < 0.00001$). Reference range for %TF was considered to be from 0 to 18.8% (mean ± 3 × SD of controls).

Statistical Analysis

Results are shown using mean ± SD. Comparisons between groups were carried out by Fisher's exact test for categorical variables, and by *t*-test for continuous variables. Adjustment for multiple comparisons was made by Bonferroni's method when appropriate.

RESULTS

Lipids and Lipoprotein Studies

IDDM patients presented lipoprotein abnormalities (Table II) with increased LDL-triglycerides ($P =$

TABLE II. Lipids and Lipoproteins in IDDM Patients and Controls†

	IDDM (n = 58)	Controls (n = 60)
Total		
Cholesterol (mmol/L)	4.96 ± 1.39	4.94 ± 0.88
Triglycerides (mmol/L)	0.89 ± 0.41	0.94 ± 0.51
Lipoprotein(a) (g/L)	0.14 ± 0.17	0.12 ± 0.15
Very low density lipoproteins		
Cholesterol (mmol/L)	0.20 ± 0.13	0.22 ± 0.06
Triglycerides (mmol/L)	0.29 ± 0.20	0.30 ± 0.21
Proteins (g/L)	0.13 ± 0.08	0.13 ± 0.07
Intermediate density lipoproteins		
Cholesterol (mmol/L)	0.14 ± 0.08	0.16 ± 0.10
Triglycerides (mmol/L)	0.12 ± 0.07	0.12 ± 0.09
Proteins (g/L)	0.05 ± 0.06	0.06 ± 0.04
Low density lipoproteins		
Cholesterol (mmol/L)	2.86 ± 0.98	2.89 ± 0.77
Triglycerides (mmol/L)	0.24 ± 0.11***	0.16 ± 0.08
Proteins (g/L)	0.54 ± 0.13	0.52 ± 0.18
High density lipoproteins		
Cholesterol (mmol/L)	1.20 ± 0.43	1.18 ± 0.44
Triglycerides (mmol/L)	0.18 ± 0.10**	0.12 ± 0.12
Proteins (g/L)	1.22 ± 0.28**	1.10 ± 0.18

†IDDM: insulin-dependent diabetes mellitus.
*P < 0.05, **P < 0.01, ***P < 0.001 compared with controls.

TABLE III. Hemostasis Parameters in IDDM Patients and Controls†

	IDDM (n = 58)	Controls (n = 60)
Prothrombin time	1.07 ± 0.21	1.03 ± 0.18
aPTT	1.03 ± 0.13	1.01 ± 0.14
Factor VII (U/mL)	90.8 ± 25.1	98.7 ± 21.9
Fibrinogen (g/L)	2.15 ± 0.89	2.42 ± 0.68
Antithrombin III (%)	98.0 ± 8.8	102.5 ± 12.9
Plasminogen (%)	106.6 ± 8.5	103.8 ± 12.0
Protein C (%)	88.6 ± 17.4*	97.0 ± 18.6
D-dimer (μg/mL)	0.96 ± 0.36***	0.17 ± 0.09
t-PA (ng/mL)	4.24 ± 1.92	4.60 ± 1.43
PAI-1 (U/mL)	13.5 ± 6.9	12.4 ± 6.6
Thrombomodulin (ng/mL)	27.9 ± 15.2	26.7 ± 10.0
F_{1+2} (nmol/L)	1.84 ± 1.37***	0.67 ± 0.28
TAT (ng/mL)	17.5 ± 28.3***	2.8 ± 2.1
Induced tissue factor expression		
MFI (afu)	17.6 ± 12.7***	8.7 ± 4.0
%TF (%)	14.4 ± 11.0***	8.3 ± 3.5

†IDDM: insulin-dependent diabetes mellitus; aPTT: activated partial thromboplastin time; t-PA: tissue-type plasminogen activator; PAI-1: type I plasminogen activator inhibitor; F_{1+2}: prothrombin fragment 1 + 2; TAT: thrombin-antithrombin III complexes; MFI: mean channel of fluorescence intensity; afu: arbitrary fluorescence units; %TF: percentage of tissue factor positive monocytes.
*P < 0.05, **P < 0.01, ***P < 0.001 compared with controls.

0.0002) and HDL-triglycerides (P = 0.004) as compared with controls.

No differences were found in lipoprotein concentrations between patients with and without clinically detectable microangiopathy.

Apo E phenotypes were distributed as follows: E 3/3 68.9%, E 3/2 22.4%, E 4/3 8.6%. This distribution did not differ from those observed in the controls (E 3/3 71.7%, E 3/2 15.0%, E 4/3 13.3%).

Hemostasis Studies

Hemostasis parameters in patients and controls are shown in Table III. IDDM patients showed a highly significant increase in F_{1+2} (P < 0.00001), TAT (P = 0.0001), and D-dimer (P < 0.00001) concentrations, and a moderate decrease in protein C (P = 0.01).

The results obtained in hemostasis parameters in patients with and without clinically detectable microangiopathy are shown in Table IV. A significant increase in thrombomodulin (P = 0.02) and PAI-1 levels (P = 0.00001) was seen in patients with clinically detectable microangiopathy, but there were no differences in TAT and D-dimer.

Induced Tissue Factor Expression on Monocytes

Incubation of plasmas from IDDM patients with mononucleated peripheral blood cells induced increased tissue factor expression on monocytes (MFI = 17.6 ± 12.7, and %TF = 14.4 ± 11.0%, P < 0.00001 and P =

TABLE IV. Hemostasis Parameters in IDDM Patients With and Without Clinically Detectable Microangiopathy†

	IDDM with microangiopathy (n = 13)	IDDM without microangiopathy (n = 45)
Prothrombin time	1.10 ± 0.15	1.06 ± 0.20
aPTT	1.02 ± 0.13	1.03 ± 0.13
Factor VII (U/mL)	94.3 ± 26.8	89.9 ± 24.7
Fibrinogen (g/L)	2.26 ± 0.74	2.11 ± 0.93
Antithrombin III (%)	99.7 ± 10.2	97.6 ± 8.4
Plasminogen (%)	105.5 ± 8.1	106.9 ± 8.6
Protein C (%)	86.9 ± 22.0	89.1 ± 16.1
D-dimer (μg/mL)	1.10 ± 0.36	0.92 ± 0.35
t-PA (ng/mL)	4.31 ± 1.85	4.21 ± 1.96
PAI-1 (U/mL)	20.3 ± 7.9***	11.5 ± 5.2
Thrombomodulin (ng/mL)	36.4 ± 23.0**	25.4 ± 11.3
F_{1+2} (nmol/L)	1.91 ± 1.45	1.82 ± 1.35
TAT (ng/mL)	15.8 ± 29.0	17.9 ± 28.4
Induced tissue factor expression		
MFI (afu)	27.3 ± 14.2***	14.7 ± 10.9
%TF (%)	22.8 ± 12.8***	12.0 ± 9.3

†IDDM: insulin-dependent diabetes mellitus; aPTT: activated partial thromboplastin time; t-PA: tissue-type plasminogen activator; PAI-1: type 1 plasminogen activator inhibitor; F_{1+2}: prothrombin fragment 1 + 2; TAT: thrombin-antithrombin III complexes; MFI: mean channel of fluorescence intensity; afu: arbitrary fluorescence units; %TF: percentage of tissue factor positive monocytes.
*P < 0.05, **P < 0.01, ***P < 0.001 compared with patients without clinically detectable microangiopathy.

0.00008, in comparison with controls, Table III). Fourteen patients (24.1%) had %TF above the reference range whereas the remaining showed normal values.

Samples from patients with clinically detectable microangiopathy had higher induced tissue factor expression (MFI = 27.3 ± 14.2, and %TF = 22.8 ± 12.8%) than samples from patients without microangiopathy (MFI = 14.7 ± 10.9, and %TF = 12.0 ± 9.3%, $P = 0.001$ and $P < 0.00001$, Table IV). The proportion of patients with %TF above the reference range was higher in patients with (8/13) than in patients without (6/45) clinically detectable microangiopathy ($P = 0.001$).

Follow-Up

Nine out of 45 (20.0%) patients without clinically detectable microangiopathy on recruitment developed retinopathy in the follow-up, but none developed nephropathy or macroangiopathy. The metabolic control of these patients had been stable during the follow-up. Patients who developed clinically detectable microangiopathy in the follow-up had significantly higher induced tissue factor expression (MFI = 23.3 ± 11.8 afu and %TF = 21.8 ± 10.1%) than patients who did not (MFI = 12.7 ± 9.8 afu and %TF = 9.7 ± 7.4%, $P = 0.008$ and $P = 0.0002$, respectively). The proportion of patients with %TF above the reference range was higher in patients who developed clinically detectable microangiopathy (5/9) than in the group that did not (1/36) ($P = 0.0006$). No significant differences were seen in the remaining hemostasis and lipoprotein variables between IDDM patients who developed clinically detectable retinopathy in the follow-up and patients who did not.

DISCUSSION

Lipoproteins and hemostatic parameters and their relationship with microvascular damage were studied in a cohort of IDDM patients controlled for BMI and with stable metabolic control. Furthermore, we prospectively studied whether patients who developed clinically detectable microangiopathy presented previously specific lipid or hemostatic abnormalities.

Lipoprotein determinations showed that, despite normal total cholesterol and triglyceride concentrations, IDDM patients had abnormal lipoprotein composition. Patients presented triglycerides enrichment of LDL and HDL, and apo E phenotype was similar in IDDM patients and controls. Lipid abnormalities are relatively common in IDDM patients [7] and are related to metabolic control, BMI, and drug administration. These abnormalities are less evident in non-obese, well-controlled, diabetic patients but qualitative lipoprotein alterations persist in these patients [22]. The present study confirms lipid abnormalities in IDDM patients but these alterations were not predictive of microvascular damage, suggesting that lipoprotein abnormalities may be a secondary or independent manifestation of a primary event. In poorly controlled IDDM, as at the onset of the disease [23], lipid parameters are extremely altered but rapidly normalize when optimal glycemic control is achieved [23,24]. However, some authors have found alterations in lipid metabolism despite optimal metabolic control [25]. This fact has been attributed to a peripheral hyperinsulinemic state, which may modify lipoprotein lipase action [26].

In the damage of small vessels, other factors, not directly related with glycemic control, may contribute to the development of microangiopathy. The present study demonstrates increased activity of PAI-1 in IDDM patients with clinically detectable microangiopathy. This PAI-1 alteration may cause a lower fibrinolytic response, predisposing the growth of thrombi and increasing the exposure of the vessel walls to clot associated mitogens. An excess of PAI-1 has been previously reported in IDDM patients [27–29], as has an increase of PAI-1 determined in vitro after cell stimulation with high concentrations of insulin [30] or glucose [31]. In addition, a stronger lysis-resistance of platelet-rich clots, attributed to a change in platelet PAI-1 activity, has been observed in IDDM [32]. However, no relationship has been found between alterations in fibrinolysis parameters and clinical findings [33] or between PAI-1 genetic polymorphism and retinopathy [34]. Elevated PAI-1 may be related to the extent of vascular damage rather than be the cause [34], and the present results, with elevated PAI-1 associated with clinically detectable microangiopathy but not with evolutive findings, support this hypothesis. Elevated thrombomodulin in patients with clinically detectable microangiopathy probably also reflect the degree of endothelial damage [35,36]. In addition, thrombomodulin is less associated with factors such as age or BMI than is PAI-1 [37,38]. For these reasons, thrombomodulin may be a promising marker for vascular evaluation in IDDM.

Hypercoagulability in IDDM is a controversial issue. We found increased thrombin generation and fibrin formation that seem to confirm a hypercoagulable state. These results agree with several reports [27,39–42] but not with others [29,43–45]. Differences in patient populations and in laboratory methods can explain some of the discrepancies. Fairly well-controlled patients seem to have less pronounced hemostasis alterations [44,46,47] but, even in the studies that did not find increased hypercoagulability markers in IDDM, some groups of patients, such as those with microalbuminuria, can present moderately elevated levels of F_{1+2} [44]. In addition, some hypercoagulability markers can be elevated and the remaining persist within the normal range [29]. At present, it is not known if these differences reflect a true different thrombin generation rate or can be explained by the different metabolism and excretion of the markers [40,44]. However, in a recent study, no elevation of hy-

percoagulable markers was found in healthy individuals with microalbuminuria [46]. In addition, in other studies, elevations in both prothrombotic and antithrombotic factors had been reported in IDDM patients with [47] and without [48] microalbuminuria. It has been hypothesized that the raised prothrombotic factors may be a microvascular risk factor but the elevation of antithrombotic factors has no protective effect [47]. These results and the enhanced anticoagulant response to activated protein C in both IDDM patients with and without microalbuminuria [44] indicate the complex regulation of hemostasis in this disease.

Our results showing a hypercoagulable state in IDDM cannot explain why only some patients had microangiopathy since elevated F_{1+2}, TAT and D-dimer were found despite the presence, or not, of clinically detectable microvascular lesions. Similar results with hypercoagulability markers have been reported in both normo and microalbuminuric IDDM patients [40,42] but others have obtained different results [49] showing increases of F_{1+2} in microalbuminuric but not in normoalbuminuric patients. These discrepancies can be attributed to the short number of patients or the patient's selection criteria [49].

The increased tissue factor expression induced by incubation of normal monocytes with plasma from IDDM patients with clinically detectable vascular lesions suggests that thrombin formation in these patients may be mediated, at least in part, by cell surface tissue factor expression. Modifications in monocyte procoagulant or tissue factor expression in IDDM have received little attention to date, but increased procoagulant activity has been found in circulating monocytes with no relationship to clinical findings [50]. In IDDM, monocytes can produce tissue factor in response to various stimuli such as alterations in the lipidic pattern [51], presence of insulin immune complexes [52], high levels of glucose [53], or presence of advanced glycation end products formed by non-enzymatic glycation of proteins [54,55]. In addition, tissue factor pathway inhibitor activity causing Xa inhibition has been described in IDDM patients with nephropathy [56], but this increase was not enough to normalize the hypercoagulability as observed by F_{1+2} elevation [56]. Elevation of tissue factor pathway inhibitor activity can be a response to increased tissue factor pathway inhibitor activity or reflect endothelial injury.

Most of the studies conducted in IDDM patients with micro- or macroangiopathy have been cross-sectional and were not able to differentiate between alterations due to vascular lesions or to be predictive of them. The present results showing increased induced tissue factor expression in asymptomatic patients who developed clinically detectable microangiopathy are interesting because impaired results were obtained prior to the development of clinical manifestations.

ACKNOWLEDGMENTS

This work was supported by grants from the Fondo de Investigaciones Sanitarias de la Seguridad Social (FIS 93/1050, FIS 94/5402 and FIS 95/0250), and Fundació Abelló i Pascual.

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